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COFC

Certificate
MAY 2 [illegible]
of Correction

May 12, 2004

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Re: Title: GENES IDENTIFIED AS REQUIRED FOR PROLIFERATION IN ESCHERICHIA COLI
Letters Patent No. 6,720,139
Issued: April 13, 2004
Our Reference: ELITRA.001A

Dear Sir:

Enclosed for filing is a Certificate of Correction in connection with the above-identified patent. In particular, this Certificate of Correction is being filed to correct the listing of inventors which appears on the face of this patent. During the prosecution of the application, Applicants filed an amendment to delete Kari L. Ohlsen, John Trawick, Jamie M. Froelich, Grant J. Carr, Robert T. Yamamoto and Howard H. Xu as inventors (amendment dated August 5, 2002 (Paper No. 26), a copy of which is enclosed herewith). The Examiner acknowledged the deletion of these individuals as inventors in his subsequent Office Action (Office Action mailed December 20, 2002 (Paper No. 28) at page 2, a copy of which is enclosed herewith). Accordingly, Applicants request that the listing of inventors in the above-identified patent be corrected to include only the two individuals who were not deleted as inventors, Judith Zyskind and R. Allyn Forsyth.

As the error cited in the Certificate of Correction was incurred through the fault of the Patent Office, no fee is believed to be required. However, please charge our Deposit Account No. 11-1410 for any fees that may be incurred with this request.

Respectfully submitted,

Knobbe, Martens, Olson & Bear, LLP

Jerry L. Hefner
Registration No. 53,009
Customer No. 20,995

Enclosure
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20 MAY 2004

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,720,139

DATED : April 13, 2004

INVENTOR(S): Zyskind et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

The cover page of the patent in the section titled Inventors (75) reads:

Inventors: Judith Zyskind, La Jolla, CA (US); Kari L. Ohlsen, San Diego, CA (US); John Trawick, La Mesa, CA (US); R. Allyn Forsyth, San Diego, CA (US); Jamie M. Froelich, San Diego, CA (US); Grant J. Carr, Escondido, CA (US); Robert T. Yamamoto, San Diego, CA (US); Howard H. Xu, San Diego, CA (US)

The cover page of the patent in the section titled Inventors (75) **should** read:

Inventors: Judith Zyskind, La Jolla, CA (US); R. Allyn Forsyth, San Diego, CA (US)

MAILING ADDRESS OF SENDER:

Jerry L. Hefner
KNOBBE, MARTENS, OLSON & BEAR, LLP
2040 Main Street, 14th Floor
Irvine, California 92614

PATENT NO. 6,720,139

May 12, 2004

No. of add'l. copies @ 50¢ per page

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20 MAY 2004

UNITED STATES PATENT AND TRADEMARK OFFICE

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/492,709	01/27/2000	Judith Zyskind	ELITRA.001A	1266

20995 7590 12/20/2002

KNOBBE MARTENS OLSON & BEAR LLP
2040 MAIN STREET
FOURTEENTH FLOOR
IRVINE, CA 92614

EXAMINER
MARSCHEL, ARDIN H

ART UNIT	PAPER NUMBER
1631	28

DATE MAILED: 12/20/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.	Applicant(s)
09/492,709	Zyskind et al.
Examiner	**Art Unit**
Ardin Marschel	1631

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *Aug 22, 2002*

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-99 and 102-118* is/are pending in the application.

4a) Of the above, claim(s) *1-34, 45-67, 78, 80-84, 94, 95, 97, and 102-111* is/are withdrawn from consideratio

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *34-44, 68, 79, 85-93, 96, 98, 99, and 112-118* is/are rejected.

7)☒ Claim(s) *100 and 101 have been canceled.* ~~is/are objected to.~~

8)☒ Claims *1-99 and 102-118* are subject to restriction and/or election requirement

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examine

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some* c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

a)☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s). (6 sheets)
4) ☒ Interview Summary (PTO-413) Paper No(s). 23
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

20 MAY 2004

Applicants' arguments, filed 8/22/02, have been fully considered but they are not deemed to be persuasive. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set presently being applied to the instant application.

In view of the papers, filed 8/22/02(Paper No. 26), as a correction of inventorship under 37 CFR § 1.48(b), the inventorship of this nonprovisional application has been changed by the deletion of KARI L. OHLSEN, JOHN TRAWICK, JAMIE M. FROELICH, GRANT J. CARR, ROBERT T. YAMAMOTO, and HOWARD H. XU.

In the REMARKS, filed 8/22/02, on page 5 applicants made a statement that they believed that claims 45, 80-93, and 97 have been withdrawn and that the statement of pending claims is in error as stated on the Office action, apparently the Office action, mailed 5/6/02. Applicants then stated that a correction should be made to include claims 45, 80-83, and 97 as pending. In response, these remarks are not understood in that all of claims 1-99 and 102-110 remain pending in the instant application as well as newly submitted claims 111-118. Only claims 100 and 101 have been canceled as of the mailing of this office action. Within this set of 116 claims, a restriction requirement with a specie election has been applied and responded to by applicants. The elections from said restriction and specie election resulted

in withdrawl from examination of claims drawn to non-elected subject matter. The remaining claims, drawn to elected subject matter are still deemed properly to be claims 34-44, 68-77, 79, 85-93, 96, 98, 99, with newly added claims 112-118. The elected subject matter is drawn to methods of identifying compounds based on sensitized cell vs. nonsensitized cell growth inhibition wherein the specie of gene product being inhibited is polypeptide gene product activity. Consideration of claim 45 reveals that it is directed to gene product activity inhibition which is non-elected RNA subject matter and is properly withdrawn from examination. Claims 80-83 and 97 (and newly submitted claim 111) are drawn to antisense nucleic acid inhibition which is reasonably interpreted as inhibition of RNA translation which also in non-elected subject matter. Thus, claims 45, 80-83, 97, and 111 remain as pending claims, but are withdrawn from consideration due to being directed to non-elected subject matter. In summary, claims 1-99 and 102-118 are instantly pending claims and claims 35-44, 68-77, 79, 85-93, 96, 98, 99, and 112-118 are under examination with the remainder of the instantly pending claims being withdrawn from consideration.

The restriction/election requirements are still deemed proper and are therefore made FINAL.

MEW MATTER REJECTION:

Claims 114-118 are rejected under 35 U.S.C. § 112, first

paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 114-118 add NEW MATTER via the added limitations directed to "candidate compound" being "a natural product extract". Written basis for this type of candidate compound as utilized in the instant invention has not been found as filed. These limitations therefore are NEW MATTER.

VAGUENESS AND INDEFINITENESS REJECTION:

Claims 35-44, 85-93, 96, 98, 99, 112-114, 117, and 118 are rejected, as discussed below, under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

It is noted that the instant claims have been amended in the preamble, such as in claim 35, to indicate that a candidate compound is not previously known to possess cell proliferation ability, but that this has not been added as an active claim step. Thus, two possible conflicting interpretations to the claims are that 1) references which disclose only the active claim steps may be prior art and 2) the preamble wording must be included in a reference for it to be prior art against such claims. Clarification via clearer claim wording is requested.

REJECTION BASED ON PRIOR ART:

The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 68, 79, 96, 98, 112, 113, 115, 116, and 118 are rejected under 35 U.S.C. § 102(b) as being clearly anticipated by Gucev et al.[Cancer Research 56(7):1545(1996)].

Gucev et al. performs a number of tests wherein antisense or sense oligonucleotides(ODNs) are added to cells along with candidate compounds for interaction with IGFBP-3 such as Retinoic acid or TGF-β2. These tests were performed as disclosed on page 1547, starting in the lefthand column, line 9, through page 1548, lefthand column, line 5. The determining of the effect of the candidate compounds compared between antisense and sense ODN inhibition is included. It is acknowledged that Retinoic acid as well as TGF-β2 are noted in the abstract as being inhibitory of cell proliferation. As noted in the above 112, second paragraph, rejection that a possible interpretation of a claim is that a reference which anticipates the actual active claim steps albeit without citing preamble practices properly anticipates the

claims.

Claims 69-77 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

The disclosure is objected to because of the following informalities:

The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code. See, for example the specification at page 19, line 25. Applicants are requested to review the entirety of the specification for such codes. Applicants are required to delete the embedded hyperlink and/or other browser-executable code. See MPEP § 608.01.

Appropriate correction is required.

No claim is allowed.

Papers related to this application may be submitted to Technical Center 1600 by facsimile transmission. Papers should be faxed to Technical Center 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notices published in the Official Gazette, 1096 OG 30 (November 15, 1988), 1156 OG 61 (November 16, 1993), and 1157 OG 94 (December 28, 1993)(See 37 CFR § 1.6(d)). The CM1 Fax Center number is either (703)308-4242 or (703)305-3014.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ardin Marschel, Ph.D., whose telephone number is (703)308-3894. The examiner can normally be reached on Monday-Friday from 8 A.M. to 4 P.M.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Woodward, Ph.D., can be reached on (703)308-4028.

Any inquiry of a general nature or relating to the status of this application should be directed to Legal Instrument Examiner, Tina Plunkett, whose telephone number is (703)305-3524 or to the Technical Center receptionist whose telephone number is (703) 308-0196.

December 20, 2002

ARDIN H. MARSCHEL
PRIMARY EXAMINER

FORM PTO-1449 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTY. DOCKET NO. ELITRA.001A	APPLICATION NO. 09/492,709
SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT	APPLICANT Zyskind, et al.	
(USE SEVERAL SHEETS IF NECESSARY)	FILING DATE January 27, 2000	GROUP 1631

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE (IF APPROPRIATE)
AM	1	US 2002/0058260 A1	05/16/02	Zyskind, et al.	435	6	

FOREIGN PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION	
							YES	NO

EXAMINER INITIAL		OTHER DOCUMENTS (INCLUDING AUTHOR, TITLE, DATE, PERTINENT PAGES, ETC.)

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EXAMINER Ardin Marschel	DATE CONSIDERED 12-20-02

*EXAMINER: INITIAL IF CITATION CONSIDERED, WHETHER OR NOT CITATION IS IN CONFORMANCE WITH MPEP 609; DRAW LINE THROUGH CITATION IF NOT IN CONFORMANCE AND NOT CONSIDERED, INCLUDE COPY OF THIS FORM WITH NEXT COMMUNICATION TO APPLICANT.

FORM PTO-1449 U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(USE SEVERAL SHEETS IF NECESSARY)

ATTY. DOCKET NO.	APPLICATION NO.
ELITRA.001A	09/492,709
APPLICANT: Zyskind, et al.	
FILING DATE: January 27, 2000	GROUP: 1631

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE (IF APPROPRIATE)
AM	1	5,874,281	02/23/99	Dattagupta, et al.	435	238	
AM	2	5,874,567	02/23/99	Smith	536	24.5	

FOREIGN PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
AM	3	WO 95/29254	11/02/95	PCT, Atkins et al.	—			

EXAMINER INITIAL		OTHER DOCUMENTS (INCLUDING AUTHOR, TITLE, DATE, PERTINENT PAGES, ETC.)

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EXAMINER	Ardin Marschel	DATE CONSIDERED	12-20-02

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FORM PTO-1449 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTY. DOCKET NO. ELITRA.001A	APPLICATION NO. 09/492,709
SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT (USE SEVERAL SHEETS IF NECESSARY)	APPLICANT Ryskind, et al.	
	FILING DATE January 27, 2000	GROUP 1631

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE (IF APPROPRIATE)
DM	1	5,353,236	10/04/94	Subbiah	364	499	
DM	2	5,869,604	02/09/99	Rousseau, et al.	530	344	
DM	3	6,077,682	06/20/00	Inouye, et al.	435	15	
DM	4	6,156,526	12/05/00	Boriack-Sjodin, et al.	435	18	

FOREIGN PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
DM.	5	WO 99/13893	03/25/99	PCT, Nielsen et al.	—	—		

EXAMINER INITIAL		OTHER DOCUMENTS (INCLUDING AUTHOR, TITLE, DATE, PERTINENT PAGES, ETC.)
DM	6	Appelt, K. 1993. Crystal structures of HIV-1 protease-inhibitor complexes. *Perspectives in Drug Discovery and Design*, 1:23-48.
	7	Bagby, et al. 1994. Unusual helix-containing Greek keys in development-specific Ca^{2+}-binding protein S. ^{1}H, ^{15}N, and ^{13}C assignments and secondary structure determined with the use of multidimensional double and triple resonance heteronuclear NMR spectroscopy. *Biochemistry*, 33:2409-2421.
	8	Bagby, et al. 1995. Solution structure of the C-terminal core domain of human TFIIB: Similarity to Cyclin A and interaction with TATA-binding protein. *Cell*, 82:857-867.
	9	Balbes, et al. 1994. "A perspective of modern methods in computer-aided drug design." In Lipkowitz, et al., Eds. Reviews in Computational Chemistry V. Chap. 7, pp. 337-379. New York: VCH Publishers.
	10	Brunschwig, et al. 1992. A two-component T7 system for the overexpression of genes in *Pseudomonas aeruginosa*. *Gene*, 111:35-41.
	11	Bugg, et al. 1993. Drugs by design: Structure-based design, an innovative approach to developing drugs, has recently spawned many promising therapeutic agents, including several now inhuman trials for treating AIDS, cancer and other diseases. *Scientific American*, Dec.:92-98.
	12	Clore, et al. 1987. Three-dimensional structure of potato carboxypeptidase inhibitor in solution: A study using nuclear magnetic resonance, distance geometry, and restrained molecular dynamics. *Biochemistry*, 26:8012-8023.
	13	Crosa, et al. 1973. Molecular relationships among the Salmonelleae. *J. Bacteriol*, 115(1):307-315.
	14	Cwirla, et al. 1990. Peptides on phage: A vast library of peptides for identifying ligands. *Proc. Natl. Acad. Sci. USA*, 87:6378-6382.
	15	Devlin, et al. 1990. Random peptide libraries: A source of specific protein binding molecules. *Science*, 249:404-406.
	16	Edwards, B. H. 1999. *Salmonella* and *Shigella* species. *Clinics Lab. Med.*, 19(3):469-487.

EXAMINER Ardin Marschel	DATE CONSIDERED 12-20-02

*EXAMINER: INITIAL IF CITATION CONSIDERED, WHETHER OR NOT CITATION IS IN CONFORMANCE WITH MPEP 609; DRAW LINE THROUGH CITATION IF NOT IN CONFORMANCE AND NOT CONSIDERED, INCLUDE COPY OF THIS FORM WITH NEXT COMMUNICATION TO APPLICANT.

Notice of References Cited

Application No.	Applicant(s)		
09/492,709	Zyskind et al.		
Examiner	Group Art Unit	Page 1 of 1	
Adam Mercer	1631		

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS
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	K					
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FOREIGN PATENT DOCUMENTS

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	P						
	Q						
	R						
	S						
	T						

NON-PATENT DOCUMENTS

*		DOCUMENT (Including Author, Title, Source, and Pertinent Pages)	DATE
	U	Gucer et al., Cancer Research, Vol. 56, No. 7, pp. 1545-1550. ✓	1996
	V		
	W		
	X		

* A copy of this reference is not being furnished with this Office action.
(See Manual of Patent Examining Procedure, Section 707.05(a).)

[CANCER RESEARCH 56, 1545–1550, April 1, 1996]

Insulin-like Growth Factor Binding Protein 3 Mediates Retinoic Acid- and Transforming Growth Factor β2-induced Growth Inhibition in Human Breast Cancer Cells[1]

Zoran S. Gucev,[2] Youngman Oh, Kevin M. Kelley, and Ron G. Rosenfeld

Department of Pediatrics, School of Medicine, Oregon Health Sciences University, Portland, Oregon 97201–3042

ABSTRACT

Retinoic acid (RA) is a potent *in vitro* inhibitor of cell proliferation in various malignant cell lines. The exact mechanisms of its actions, however, are not fully understood. To further elucidate the nature of this inhibition, we investigated the effects of RA in an estrogen receptor-negative human breast cancer cell line, MDA-MB-231. RA (0.01–5 μM) significantly inhibited MDA-MB-231 cell growth by 35–40% as compared with untreated controls. Similar growth inhibitory actions were observed when cells were treated with transforming growth factor β2 (TGF-β2), another factor with antiproliferative actions in breast cancer cells.

Both RA and TGF-β2 increased the levels of insulin-like growth factor binding protein (IGFBP) 3 (2–3-fold) and mRNA (1.5–2-fold), whereas IGFBP-4 levels remained essentially unchanged. The direct involvement of IGFBP-3 in cell growth inhibition was further confirmed by its action on cell growth: exogenous IGFBP-3 directly and significantly inhibited MDA-MB-231 cell number by 40%. These results provided circumstantial evidence that IGFBP-3 may mediate RA and TGF-β2 growth inhibitory actions in human breast cancer cells.

To test this hypothesis, we used an antisense IGFBP-3 oligodeoxynucleotide (ODN) which specifically inhibits IGFBP-3 expression. The antisense IGFBP-3 ODN dramatically blocked both RA- and TGF-β2-induced increases in IGFBP-3 protein (90%) and mRNA levels (90%). This effect was not observed when RA- or TGF-β2-exposed cells were treated with sense IGFBP-3 ODN. Moreover, antisense ODN did not significantly affect IGFBP-4 protein or mRNA levels, strongly supporting the specificity of the antisense IGFBP-3 ODN effect on IGFBP-3 mRNA.

This specific effect of antisense IGFBP-3 ODN on IGFBP-3 protein and mRNA levels was accompanied by significant attenuation of the inhibition of cell proliferation attained with RA or TGF-β2 (approximately 40% of either RA- or TGF-β2-induced inhibition). The control sense IGFBP-3 ODN did not reduce the growth inhibition observed with either RA or TGF-β2.

These results indicate that IGFBP-3 is an important mediator of RA- and TGF-β2-induced cell growth inhibition in human breast cancer cells.

INTRODUCTION

IGFs[3] are potent mitogens for a wide variety of cells, including normal and malignant cell types (1). Both IGF-I and IGF-II have significant homology with insulin and exert their actions through three types of receptors: type 1 and type 2 IGF receptors, and, with lower binding affinity, through insulin receptors (2). IGFs, additionally, have high affinity for a family of binding proteins (IGFBPs). Six binding proteins (IGFBP 1–6) have been cloned and sequenced. Recently, a seventh IGFBP candidate (mac25) was identified in senescent human mammary epithelial cells (3).

Breast cancer cell lines synthesize a number of IGFBPs (4). ER^+ cell lines secrete IGFBP-2 as a predominant IGFBP, whereas the ER^- cell lines, which generally have higher proliferation rates, produce IGFBP-3 as their major IGFBP (5, 6). The activity of the IGFs is modulated by the presence of secreted IGFBPs. In the majority of cell systems, IGFBPs inhibit the stimulatory effect of IGFs on cellular proliferation, largely as a result of sequestration of IGFs in the cellular microenvironment. However, under certain conditions, IGFBPs can potentiate IGF action (7). It has been further demonstrated that IGFBPs can modulate cellular function in the absence of IGFs (8). This phenomenon may be explained by the existence of an IGFBP-3 receptor(s) on the cell surface of Hs578T breast cancer cells, which may mediate the IGF-independent actions of IGFBP-3 (9).

Previous studies demonstrated that RA is a potent antiproliferation factor in ER^- and ER^+ human breast cancer cells (10, 11). RA also induces an increase of IGFBP-3 protein and mRNA levels (10). Recently, a major consensus sequence for RA was identified in the 5′ untranslated flanking region of the rat *IGFBP-3* gene (12). Moreover, we have recently demonstrated that TGF-β2-induced cell growth inhibition in Hs578T breast cancer cells was mediated, at least in part, by IGFBP-3 (13). This has led to speculation that IGFBP-3 may mediate RA-induced cell growth inhibition as well.

To investigate this hypothesis, we have designed and used an antisense IGFBP-3 ODN which blocks IGFBP-3 synthesis. Using this experimental approach, we now demonstrate that IGFBP-3 mediates RA-induced cell growth inhibition in the MDA-MB-231 human breast cancer cell line. We also show that IGFBP-3 mediates TGF-β2-induced cell growth inhibition in the same cell line, suggesting that regulation of the *IGFBP-3* gene transcription may be a common pathway whereby various growth factors modulate replication of cancer cells.

MATERIALS AND METHODS

Peptides and Proteins. Recombinant human IGF-I was purchased from Bachem (Torrance, CA) and recombinant IGF-II was provided by Eli Lilly (Indianapolis, IN). Recombinant human nonglycosylated IGFBP-3 and recombinant TGF-β2 were generous gifts from Celtrix, Inc. (Santa Clara, CA). All-*trans* RA was purchased from Sigma (St. Louis, MO). Iodination of IGF-I and IGF-II was performed by a modification of the chloramine-T technique, to a specific activity of 350–500 μCi/μg.

The antisense and sense phosphorothioate ODNs were purchased from Oligos Etc., Inc (Guilford, CT). The sequence of the IGFBP-3 antisense ODN designed for these experiments was 5′-CATGACGCCTGCAACCGGGG-3′ (positions 2021–2040; Ref. 13). This sequence was complementary to 20 nucleotides located immediately 5′ to and including the ATG translation initiation site (14). The sequence of the sense IGFBP-3 ODN was 5′-CCCCGGTTGCAGGCGTCATG-3′. A search of GenBank revealed no identical sequences in any other known genes.

Cell Cultures. MDA-MB-231 human breast cancer cells were maintained in DMEM supplemented with 4.5 g/liter glucose, 110 mg/liter sodium pyruvate, and 10% fetal bovine serum.

Preparation of CM. Cells were grown until 95% confluent in media containing 10% fetal bovine serum, and then switched for 12 h to serum-free DMEM. Medium was aspirated again, and cells were cultured in serum-free

Received 11/6/95; accepted 1/25/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by Grant CA58110 from NIH (R.G.R.) and by Soros Open Society Foundation Grant 78 (Z. S. G.).

[2] To whom requests for reprints should be addressed, at Department of Pediatrics, L343, School of Medicine, Oregon Health Sciences University, Portland, OR 97201–3042. Phone: (503) 394-1925; Fax: (503) 494-1933.

[3] The abbreviations used are: IGF, insulin-like growth factor; IGFBP, IGF binding protein; ER, estrogen receptor; RA, retinoic acid; TGF-β, transforming growth factor β; ODN, oligodeoxynucleotide; CM, conditioned medium.

DMEM, with or without combinations of TGF-β2, RA, and antisense or sense IGFBP-3 ODN, as described in "Results." CM were collected and centrifuged at 1000 × *g* for 10 min at 4°C to remove cell debris. The harvested CM from triplicate wells within each experiment were pooled and stored at −70°C until assay.

Western Ligand Blots. Proteins from CM were subjected to Western ligand blot analysis, as described by Hossenlopp *et al.* (15). Samples were diluted with nonreducing SDS-dissociation buffer [0.5 M Tris (pH 6.8), 69% glycerol, and 4% SDS], loaded onto a 1.5-mm discontinuous SDS polyacrylamide gel, and electrophoresed through a 4% stacking gel and 12% separating gel at 50 V overnight. Electrotransfer of the proteins from the gels to 0.45 μm nitrocellulose (Schleicher and Schuell, Keene, NH) was performed using a Hoefer Semi-Dry Transfer unit (San Francisco, CA). Posttransfer, nitrocellulose sheets were initially washed in NP40 (3%, v/v) for 30 min, followed by blocking in BSA (1%, w/v in Tris-buffered saline) for 2 h, and Tween 20 (0.1%, v/v) for 15 min. Nitrocellulose filters were probed with a combination of ^{125}I-radiolabeled IGF-I and IGF-II (1 × 10^6 cpm each) in Tris-buffered saline overnight. Nitrocellulose papers were then washed extensively in Tween 20 (1%, v/v), dried, and exposed to X-ray film (Kodak X-Omat AR; Eastman Kodak Co., Rochester, NY) in the presence of Cornex Hi-Plus Intensifying Screens (Du Pont, Wilmington, DE) for 3 to 7 days at −70°C. Band densities were quantified on an LKB denistometer.

Monolayer Cell Replication Assay. Cells were grown in 12-multiwell plates until 60% confluency (0.2 × 10^6 cells/well) and then changed to serum-free DMEM for 12 h. Cells were then cultured for 5 days in serum-free DMEM containing 0.25% BSA in the presence or absence of different concentrations of TGF-β2, RA, or IGFBP-3. PBS-EDTA was used to gently detach cells from the plates, and cell number was counted using a Coulter Z1 cell counter (Coulter, Ltd., Beds, England). The effect of antisense or sense IGFBP-3 ODN (0.01–20 μg/ml) on cell proliferation was performed in the presence of 5 ng/ml TGF-β2 or 0.5 μM RA for 5 days.

Northern Blots. Total cellular RNA was isolated from cells (treated for 24 h) by a modification of the guanidinium isothiocyanate precipitation method (16). RNA samples were size fractionated on 1.2% agarose-formaldehyde gels and then transferred to Zeta probe membranes (Bio-Rad, Richmond, CA). Blotted RNAs were hybridized with cDNA probes labeled with [^{32}P]dCTP by random oligo priming (Prime-It; Stratagene, La Jolla, CA). Bands were visualized using autoradiography after exposure to XAR film with intensifying screens at −70°C. RNA ladder size markers (Bethesda Research Laboratories, Grand Island, NY) were used to obtain size estimates of the specific transcripts. A 1082-bp *Eco*RI-*Pvu*II 5′ fragment of the human IGFBP-3 cDNA, which includes the entire coding region sequence, was used as a probe for human IGFBP-3 mRNA (17). A 440-bp *Sma*I-*Sma*I restriction fragment of the 1.2-kb IGFBP-4 cDNA isolated from a human osteosarcoma cell gene library (a generous gift from Dr. S. Mohan, Loma Linda, CA) was used as a probe for human IGFBP-4 mRNA; Ref. 18). An 18S rRNA probe was used on each Northern blot to serve as an internal control. Band densities were quantified on an LKB denistometer and normalized to corresponding values for 18S rRNA bands.

Statistical Analysis. Data were analyzed with a two-tailed Student' *t* test; using the software program, Statview (Abacus Concept, Inc.).

RESULTS

When MDA-MB-231 cells were seeded at low concentrations, serum starved and treated for 5 days, RA (0.01–5 μM) inhibited cell growth in a dose-dependent manner, with maximum inhibition (40%; $P < 0.05$) at 5 μM (Fig. 1*A*). Dose-dependent inhibition of cell growth was also observed with 5 days of TGF-β2 treatment (35%; $P < 0.05$), in doses ranging from 0.5 to 5 ng/ml (Fig. 1*B*).

The effect of RA or TGF-β2 on IGFBPs secreted into the CM was examined. Two species of binding proteins were detected: IGFBP-3 (M_r 39,000 and 41,000) and a doublet of IGFBP-4 (M_r 24,000 nonglycosylated and 28,000 glycosylated forms). Confirmation of the identity of these IGFBPs as IGFBP-3 and IGFBP-4 was performed by immunoblotting and deglycosylation (data not shown). Treatment with 0.5 μM RA for 3 days increased the concentration of IGFBP-3 protein in CM by 3.0-fold (mean ± SD, 3.06 ± 0.87; Fig. 2*A*).

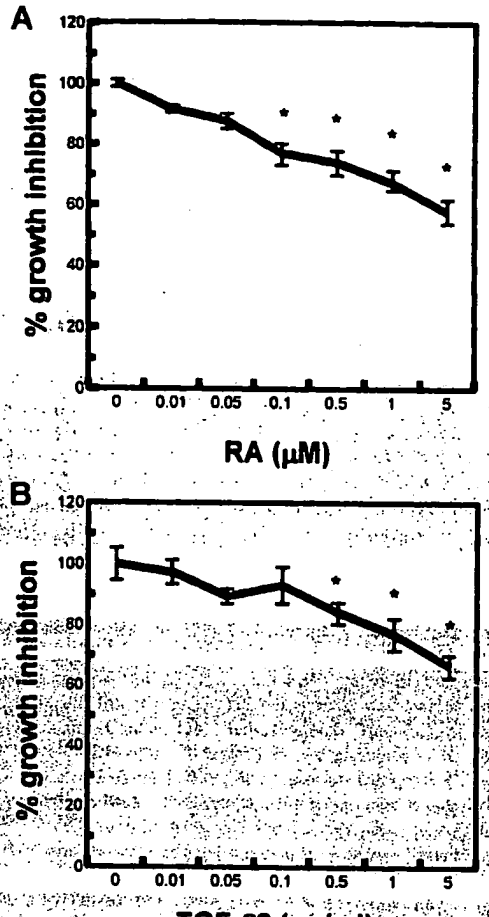

Fig. 1. Effect of RA (*A*) or TGF-β2 (*B*) on MDA-MB-231 cell growth. MDA-MB-231 cells were grown until 60% confluent, (0.2 × 10^6 cells/well), and then changed to serum-free media containing 0.25 BSA with various concentrations of RA (0.01–0.5 μM) or TGF-β2 (0.01–5 ng/ml). Cell numbers were counted with a Coulter Z1 counter (Coulter, Ltd.) after 5 days of treatment. Results represent the means of two separate experiments performed in triplicate. The two-tailed *t* test was used. * $P < 0.05$. Data are expressed as the percentage of control cell numbers at each RA or TGF-β2 concentration. *Bars*, SE.

Treatment with 5 ng/ml TGF-β2 for 3 days increased IGFBP-3 protein levels in CM by 2–3.0-fold (mean ± SD, 2.57 ± 0.48; Fig. 2*B*). Although, the M_r 24,000–28,000 forms of IGFBP-4 were slightly increased in this experiment, when multiple studies were evaluated, no significant increase was observed.

Since it has been demonstrated that IGFBP-3-specific proteases are important regulators of secreted and cell surface-bound IGFBP-3 levels (19, 20), CM from MDA-MB-231 cells were also tested for IGFBP-3 protease activity according to the method of Lamson *et al.* (19). In this cell system, no IGFBP-3 protease activity was detectable in CM, either from control cells or from cells treated with RA or TGF-β2 (data not shown).

To assess whether changes in IGFBP-3 protein levels were accompanied by changes in gene expression, total RNA was extracted from the MDA-MB-231 cells after treatment with 0.5 μM RA or 5 ng/ml TGF-β2 (Fig. 2*C*). Treatment with RA resulted in a 2-fold increase in IGFBP-3 mRNA levels (mean, 2.16). IGFBP-3 mRNA was increased 1.5–2-fold (mean, 1.82) by TGF-β2. Both antiproliferative factors did not significantly affect IGFBP-4 protein or mRNA levels. Thus, the observed increase in the IGFBP-3 protein concentrations in CM coincided with changes in steady-state mRNA levels.

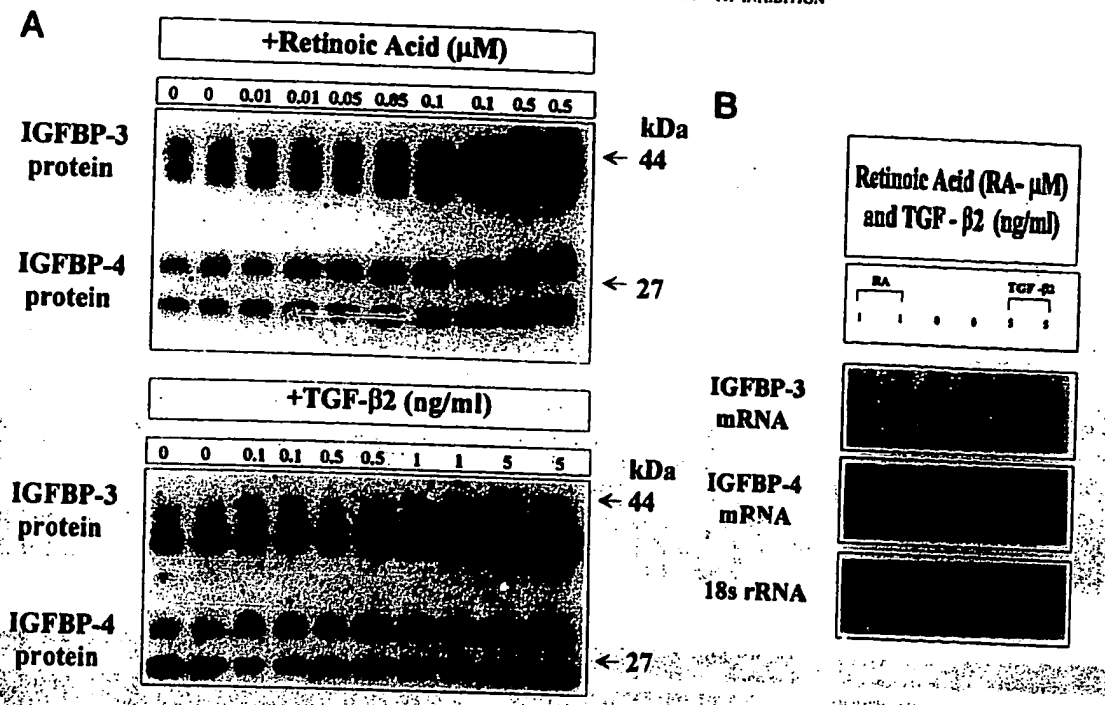

Fig. 2. Effect of RA (*A*) or TGF-β2 (*B*) on IGFBP-3 and IGFBP-4 concentrations in CM of MDA-MB-231 cells. Representative Western ligand blots of CM from cells incubated in DMEM alone or with either 0.01–0.5 μM RA (*A*) or 0.01–5 ng/ml TGF-β2 (*B*). CM harvested from triplicate wells within each experiment were pooled and size fractionated using SDS-PAGE and electroblotted onto nitrocellulose filters. A representative gel from one of three experiments is shown. A densitometric analysis of the bands was performed. *C*, effect of RA or TGF-β2 on IGFBP-3 and IGFBP-4 mRNA levels. Cells were grown until 90% confluent and incubated in serum-free media or serum-free media containing 0.5 μM RA or 5 ng/ml TGF-β2. Total RNA was extracted after 24 h and analyzed using Northern blots. A representative blot from two separate experiments is shown. *Lanes 1* and *2*, RA-treated cells; *Lanes 3* and *4*, untreated cells; and *Lanes 5* and *6*, TGF-β2-treated cells.

The effect of exogenous recombinant human nonglycosylated IGFBP-3 on cell growth is shown in Fig. 3. Treatment of cells with 0.5–20 nM recombinant human IGFBP-3 for 5 days resulted in a dose-dependent reduction in cell number, with a 40% decrease at 20 nM IGFBP-3. Since MDA-MB-231 cells are IGF-I negative (21), this cell growth inhibition could not be attributed to the formation of IGF-I-IGFBP-3 complexes, but indicated an IGF-independent action of IGFBP-3 on cell proliferation.

An antisense ODN strategy was used to investigate whether IGFBP-3 mediates RA and TGF-β2 inhibition of MDA-MB-231 cells. We first assessed the effect of IGFBP-3 ODN on basal IGFBP-3 levels. Treatment with 0.01–20 μg/ml antisense, but not sense, reduced basal IGFBP-3 concentrations in CM by 80% (data not shown). At the same time, antisense and sense ODNs did not significantly affect IGFBP-4 levels. When cells were treated with RA (0.5 μM), IGFBP-3 mRNA and protein increased 2–3-fold with RA alone (mean, 2.49 and 2.21, respectively), but this increase was markedly inhibited by the simultaneous addition of antisense ODN (Fig. 4, *A* and *B*). The antisense IGFBP-3 ODN inhibition of the RA-induced increase in IGFBP-3 protein increase was observed at ODN concentrations of 0.05–20 μg/ml and exceeded 90% inhibition at the antisense ODN concentration of 1 μg/ml. IGFBP-4 concentrations were not affected by the antisense IGFBP-3 ODN. Additionally, treatment of cells with the sense IGFBP-3 ODN had no effect on RA-induced increases in IGFBP-3 mRNA (Fig. 4*A*) or protein (Fig. 4*C*).

When cells treated with 5 ng/ml TGF-β2 were exposed to antisense IGFBP-3 ODN, the antisense inhibitory effect on IGFBP-3 mRNA and protein was observed at ODN concentrations of 0.01 μg/ml and was maximum (>90%) at 20 μg/ml antisense IGFBP-3 ODN (Fig. 5, *A* and *B*). IGFBP-4 mRNA as well as protein concentrations in CM were unaffected by treatment with the antisense IGFBP-3 ODN. Treatment with sense IGFBP-3 ODN had no effects on IGFBP-3 mRNA (Fig. 5*A*) or protein (Fig. 5*C*).

The ability of IGFBP-3 antisense and sense ODN to block the antiproliferative effects of RA or TGF-β2 was evaluated. To investigate whether the antisense and sense ODN have growth effects themselves, the cells were treated with 0.01–20 μg/ml ODN alone for 5 days. No significant change was observed in the cell number (data not shown). However, antisense, but not sense ODN attenuated the RA- or TGF-β2-induced cell growth inhibition by approximately 40%

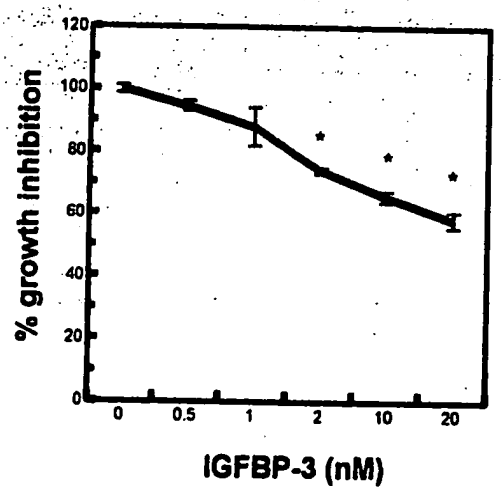

Fig. 3. Effect of exogenous recombinant IGFBP-3 on MDA-MB-231 cell growth. Cells cultured in DMEM alone were treated with IGFBP-3 at concentrations between 0.5 and 20 nM. Results represent the means of three separate experiments performed in triplicate. *, $P < 0.05$. *Bars*, SE.

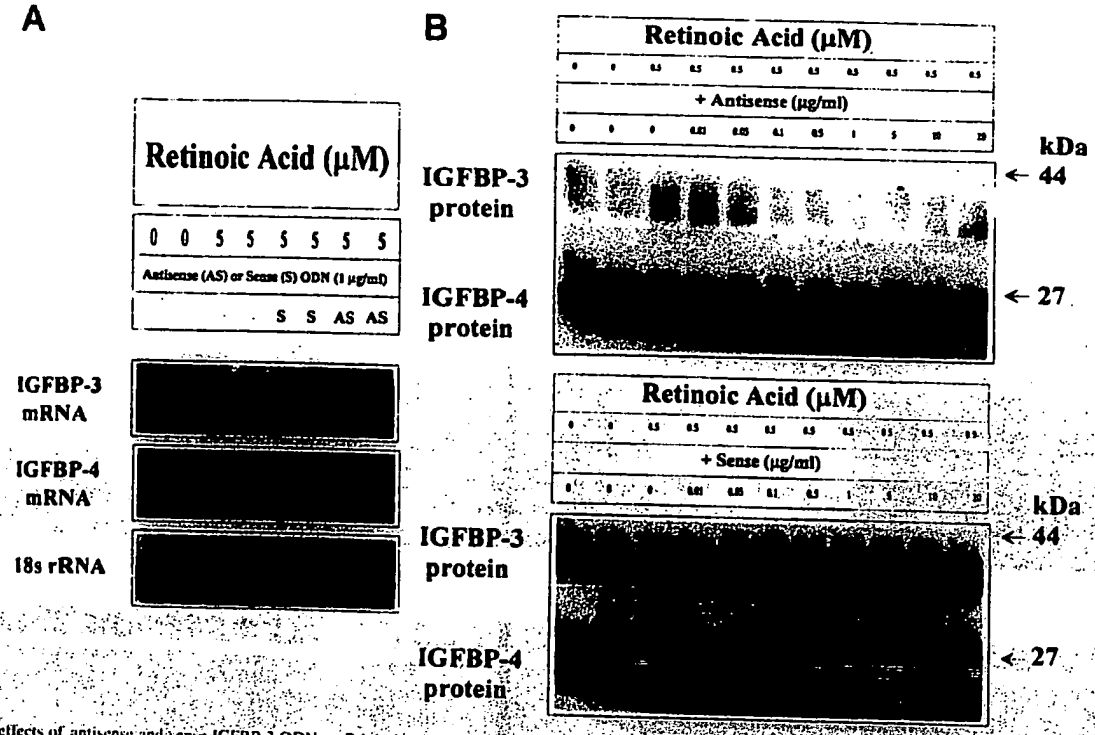

Fig. 4. *A*, effects of antisense and sense IGFBP-3 ODN on RA-induced IGFBP-3 and IGFBP-4 mRNA levels. Cells were grown until 90% confluent and incubated in serum-free media containing 0.5 μM RA; 0.5 μM RA with 1 μg/ml antisense IGFBP-3; or 0.5 μM RA with 1 μg/ml IGFBP-3 sense ODN. Total RNA was extracted after 24 h and analyzed using Northern blots. Duplicate consecutive lanes represent: IGFBP-3 mRNA in controls, IGFBP-3 mRNA in cells treated with 0.5 μM RA, IGFBP-3 mRNA from cells treated with 0.5 μM RA and 1 μg/ml sense ODN, and, the last two lanes show IGFBP-3 mRNA from cells treated with 0.5 μM RA and 1 μg/ml antisense IGFBP-3 ODN. A representative blot from two separate experiments is shown. Effect of antisense (*B*) or sense (*C*) IGFBP-3 ODN on IGFBP protein levels in control and RA-treated cells. Western ligand blots of MDA-MB-231 CM from cells treated with 0.5 μM RA and 0.01–20 μg/ml IGFBP-3 antisense (*B*) or sense (*C*) ODN. The first two lanes show CM from cells incubated in DMEM alone, the third lane represents CM from cells treated with 0.5 μM RA, and the succeeding lanes show CM from cells incubated with 0.5 μM RA and antisense (*B*) or sense (*C*) ODN in concentrations of 0.01–20 μg/ml. CM were harvested after 3 days of treatment from triplicate wells within each experiment, pooled, and electrophoresed using 12 SDS-PAGE under nonreducing conditions. Representative Western ligand blots from two experiments are shown.

($P < 0.05$; Fig. 6). Maximum reduction of cell growth inhibition was attained at antisense IGFBP-3 ODN concentrations of 0.5 μg/ml (RA) and 10 μg/ml (TGF-β2). Treatment with sense IGFBP-3 ODN did not significantly affect the RA- or TGF-β2-induced cell growth inhibition.

DISCUSSION

Several growth factors, including IGFs, TGFs, epidermal growth factor, platelet-derived growth factor, and fibroblast growth factor, are known to have important growth regulatory roles in normal and malignant breast epithelia (22). That IGFs and their binding proteins are important regulators of proliferation in breast cancer cells has been amply demonstrated (1). The elucidation of their mechanisms of action is rendered difficult, however, by the multitude of IGF ligands and binding proteins and the complex interactions existing in the IGF system. Multiple IGFBPs have been identified in human breast cancer cell lines, where they are believed to modulate IGF access to target receptors (1, 3–5, 8–10) and also exert IGF-independent actions (8, 9, 13).

Retinoids, particularly RA, markedly inhibit *in vitro* proliferation of breast cancer cells (23, 24). This inhibition is especially pronounced in ER^+ breast cancer cell lines and is thought to be due to retinoid antagonization of estrogen stimulation. In ER^- breast cancer cell lines, the mechanism of RA-induced cell growth inhibition is even more obscure.

Several observations have suggested that IGFBP-3 may be an important mediator of RA actions in breast cancer cells. Treatment with RA resulted in a greater than 3-fold increase in secreted IGF-binding activity and induced the appearance of M_r 42,000 and 46,000 IGFBPs on ligand blotting (11). This RA effect on IGFBP secretion was accompanied by inhibition of cellular growth and arrest of the cells in G_1. Similarly, treatment of MDA-MB-231 and MDA-MB-468 human breast cancer cells with RA increased cellular IGFBP-3 mRNA levels, as well as IGFBP-3 release into the CM, and also caused cell growth inhibition (10). Since RA-inhibited cell growth occurred coincidentally with increased IGFBP-3 protein and mRNA levels, its involvement in breast cancer cell growth inhibition has been suspected (10, 11). This hypothesis is supported by the demonstration that a major consensus sequence for RA exists in the promoter of the rat *IGFBP-3* gene (12).

We have previously demonstrated that exogenous IGFBP-3 inhibits growth in Hs578T breast cancer cells (8). It was also shown that in the ER^+ MCF-7 breast cancer cells, treatment with IGFBP-3 significantly inhibited cell proliferation (25). In this study, exogenous IGFBP-3 also inhibited growth in MDA-MB-231 cells (~40%). Furthermore, the cell growth inhibition resulting from treatment with RA was accompanied by increased IGFBP-3 protein (mean ± SD, 3.06 ± 0.87) and mRNA (mean, 2.16) levels. That the increased expression of IGFBP-3 was mediating, at least in part, the inhibitory effects of RA was confirmed by experiments using the antisense IGFBP-3 ODN. Treatment with the antisense IGFBP-3 ODN blocked the increased IGFBP-3 expression and reversed by 40% the cell growth inhibition induced by RA. Sense IGFBP-3 ODN, on the other hand, neither suppressed the IGFBP-3 protein or mRNA level nor reduced the cell growth inhibition achieved with RA. This apparent specificity of action of the antisense IGFBP-3 ODN was further

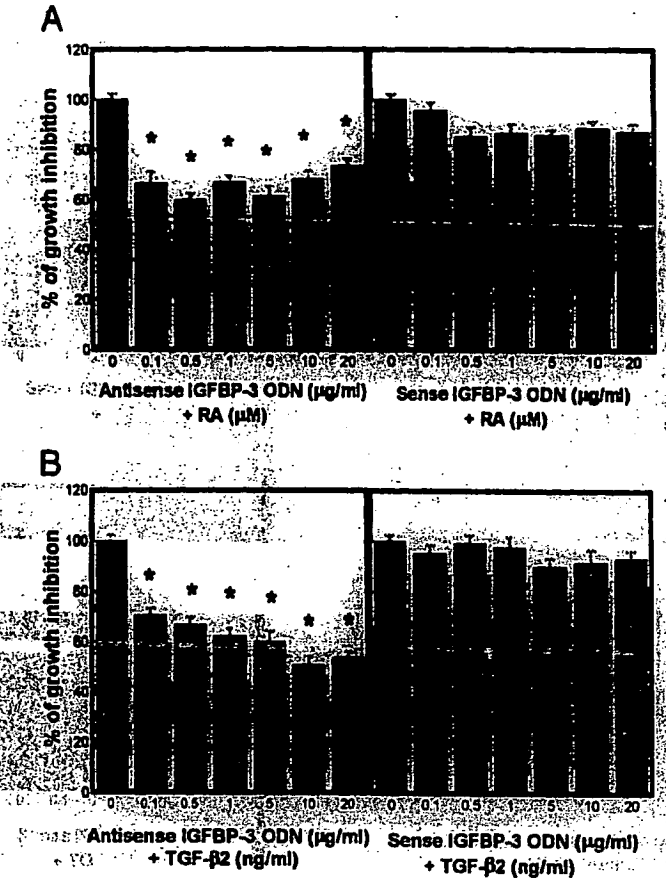

Fig. 6. Effect of antisense and sense IGFBP-3 ODN on RA- (*A*) or TGF-β2- (*B*) induced cell growth inhibition. Cells were grown until 60% confluent and than treated with 0.5 μM RA (*A*) or 5 ng/ml TGF-β2 in the presence or absence of ODN at concentrations ranging from 0.1 to 20 μg/ml. The growth inhibitory effects of RA or TGF-β2 and ODN were determined by cell counts after 5 days of treatment. The first lane in each graph shows inhibition of cell growth by RA or TGF-β2 alone, and has been given an arbitrary value of 100inhibition. Results represent the means of two separate experiments performed in triplicate. $^{*}P < 0.05$. *Bars*, SE.

anism by which multiple growth factors modulate breast cancer growth.

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APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.

EXAMINER	
ART UNIT	PAPER NUMBER
	23

DATE MAILED:

INTERVIEW SUMMARY

All participants (applicant, applicant's representative, PTO personnel):

(1) DANIEL HART (appl rep) (3) JERRY HEFNER (appl rep)

(2) ARDIN MARSCHEL (Exr) (4) JEFF WINKELMAN (assignee rep)

Date of Interview 7-25-02

Type: ☒ Telephonic ☐ Televideo Conference ☐ Personal (copy is given to ☐ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☒ No If yes, brief description: _____

Agreement ☐ was reached. ☒ was not reached.

Claim(s) discussed: 35, 96, and all others under examination generically

Identification of prior art discussed: Mutter et al.

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: We discussed possible claim limitations to overcome all the refs. of record.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

☒ It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has been checked to indicate to the contrary. A FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has are ready been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

Examiner Note: You must sign this form unless it is an attachment to another form.

UTILITY/DESIGN PATENT
(amend/~~final amend/appeal~~)

Date: 8-5-02
Date of Action: 5-6-02

Rec'd in the USPTO on the date stamped hereon via Certificate of Mail:
Docket #: ELITRA.001A Applicant:
Title: Genes Identified as Required for Proliferation
App No.: 09/492,709 Filed: 1-27-00
VERIFIED BY: Asst: dlr Atty: QC: DOU

- ☒ Transmittal Letter
- ☒ Amendment in 11 pgs
- ☐ ____ Month Extension Requested
- ☒ Information Disclosure Statement; with PTO-1449 w/ 1 Ref(s)
- ☐ Terminal Disclaimer in ____ pgs
- ☐ Sequence Submission Statement
- ☐ Sequence Listing in ____ pgs
- ☐ ____ copies of CRF Containing Seq List
- ☐ ____
- ■ Return Prepaid Postcard

- ☐ Req for Drawing Changes/Corrections
- ☐ ____ sheets of RED-Lined Drawings
- ☐ Notice of Appeal in Duplicate
- ☐ Appeal Brief in ____ pgs in Triplicate
- ☐ Request for Oral Hearing
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INTELLECTUAL PROPERTY LAW

2040 MAIN STREET
14TH FLOOR
IRVINE, CA 92614

UTILITY/DESIGN PATENT
(amend/~~final amend/appeal~~)

Date: 8-5-02
Date of Action: 5-6-02

Rec'd in the USPTO on the date stamped hereon via Certificate of Mail:

Docket #: ELITRA.009 Applicant:
Title: Genes Identified as Required for Proliferation
App No.: 09/492,709 Filed: 1-27-00
VERIFIED BY: Asst: dlr Atty: QC: DoV

- ☒ Transmittal Letter
- ☒ Amendment in 11 pgs
- ☐ ____ Month Extension Requested
- ☒ Information Disclosure Statement; with PTO-1449 w/ 1 Ref(s)
- ☐ Terminal Disclaimer in ____ pgs
- ☐ Sequence Submission Statement
- ☐ Sequence Listing in ____ pgs
- ☐ ____ copies of CRF Containing Seq List
- ☐ ____
- ■ Return Prepaid Postcard
- ☐ Req for Drawing Changes/Corrections
- ☐ ____ sheets of RED-Lined Drawings
- ☐ Notice of Appeal in Duplicate
- ☐ Appeal Brief in ____ pgs in Triplicate
- ☐ Request for Oral Hearing
- ☒ $ 310 Check for Filing Fees
- ☒ Amendment and fee to correct Inventorship under 37CFR §1.48(b)
- ☐ ____

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FOR 09/492709 JLH/DOH

Diane Epstein

MP

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Zyskind, et al.
Appl. No.	:	09/492,709
Filed	:	January 27, 2000
For	:	GENES IDENTIFIED AS REQUIRED FOR PROLIFERATION IN ESCHERICHIA COLI
Examiner	:	A. Marschel
Group Art Unit	:	1631

I hereby certify that this correspondence and all marked attachments are being deposited with Federal Express for Standard Overnight delivery to: Examiner Ardin Marschel, Art Unit 1631, 7th Floor Receptionist, United States Patent and Trademark Office, 1911 S. Clark Place, Crystal Mall, Arlington, VA 22202, on

August 5, 2002
(Date)

Daniel Hart, Reg. No. 40,637

TRANSMITTAL LETTER

Examiner Ardin Marschel
Art Unit 1631
7th Floor Receptionist
United States Patent and Trademark Office
1911 S. Clark Place, Crystal Mall
Arlington, VA 22202

Sir:

Transmitted herewith are amendments in the above-identified application.

The fee has been calculated as shown below:

CLAIMS AS FILED

	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE	ADDITIONAL FEE
Total Claims	46	—	111	= 0 ×	$ 9	= $0
Independent Claims	5	—	40	= 0 ×	$ 42	= $0
If application has been amended to contain multiple dependent claim(s), then add					$140	= $0
Time Extension Fee						$0
TOTAL ADDITIONAL FEE FOR THIS AMENDMENT						$0

(X) Amendment and Fee to Correct Inventorship under 37 C.F.R. §1.48(b).

(X) A Supplemental Information Disclosure Statement.

(X) A PTO Form 1449 with one (1) reference.

(X) A check is enclosed in the amount of $310 to cover the fees set forth in 37 C.F.R. §1.17(i) and 37 C.F.R. § 1.17(p).

(X) The present application qualifies for small entity status under 37 C.F.R. § 1.27.

(X) The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Account No. 11-1410.

(X) Return prepaid postcard.

Daniel Hart
Registration No. 40,637
Attorney of Record

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ELITRA.001A

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Zyskind, et al.	) Group Art Unit 1631
Appl. No.	:	09/492,709	)
Filed	:	January 27, 2000	)
For	:	GENES IDENTIFIED AS REQUIRED FOR PROLIFERATION IN ESCHERICHIA COLI	)
Examiner	:	Marschel, A.	)

AMENDMENT AND FEE TO CORRECT INVENTORSHIP UNDER 37 C.F.R. § 1.48(b)

Examiner Ardin Marschel
Art Unit 1631
7th Floor Receptionist
United States Patent and Trademark Office
1911 S. Clark Place, Crystal Mall
Arlington, VA 22202

Dear Sir:

This amendment is to delete the names of individuals who were originally properly named as inventors in the declaration that was filed on May 16, 2000, but whose invention is no longer being claimed in this application.

Deletion of Inventors

Please delete the following previously named individuals as inventors because their invention is no longer being claimed in this application:

KARI L. OHLSEN

JOHN TRAWICK

JAMIE M. FROELICH

GRANT J. CARR

ROBERT T. YAMAMOTO

HOWARD H. XU

This amendment is accompanied by the fee set forth in 37 C.F.R. § 1.17(i). Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: Aug 5, 2002

By: _____

Daniel Hart
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